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The effects of hypergravity on development of the heart and behavior of Xenopus laevis

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THE EFFECTS OF HYPERGRAVITY ON DEVELOPMENT OF THE HEART AND BEHAVIOR OF *XENOPUS LAEVIS*

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Abstract

Abstract

Every living organism on earth has developed and evolved in a 1G environment. It is likely that any deviation from Earth's standard gravity will influence development, particularly at early stages. Previous reports from this lab showed that body length is reduced and that ventricle size is increased during development at 7G. The objective of the present study was to investigate the effect of hypergravity on the development of the ventricular myocardial wall and neuromuscular responsiveness of *Xenopus laevis*. At early gastrulation, (Nieuwkoop and Faber Stage 10) embryos were placed in a centrifuge simulating 7G, 10G, 15G or 17G until they reached stage 45 (approximately 72 hours from initiation of gastrulation). Mortality did not differ from controls in 7G, 10G and 15G experiments and averaged less than 15%. However, exposure to 17G resulted in 100% mortality. Immediately following centrifugation, embryos were stimulated to test neuromuscular responsiveness. Thirty percent, 25%, 36%, and 57% of hypergravity embryos in 7G, 10G, and 15G, respectively, required more than a tail touch, compared to only 8% in controls. A quarter of the embryos were fixed in paraformaldehyde and their body dimensions were measured. The snout-vent proportion of body length in the 7G, 10G, and 15G groups averaged 7.4, 18.9 and 27 percent greater than control. Fixed embryos were sectioned and stained using hematoxylin. The thickness of the ventricle wall was 5.3%, 31.7%, and 61.7% greater in 7G, 10G and 15G experimental groups compared to control groups. The length of the papillary muscle was 24.8%, 49.7%, and 107% greater in 7G, 10G and 15G experimental groups as compared to control groups. The papillary muscle area was 17.7%,

37.5%, and 90.6% greater in7G, 10G and 15G experimental groups. Remaining live embryos were saved and swimming behavior was observed daily for 5 minutes. Abnormal swimming behavior was found in 33% of 7G embryos and 2% of controls during this time. These embryos were later subjected to an orientation-swimming test. Hypergravity embryos required, on average, 9% longer to become oriented. The data demonstrates that hypergravity has significant effects on the development of the ventricular myocardial wall, neuromuscular responsiveness, and equilibrium organs. Specific mediators of these apparent effects on cardiac tissue and swimming behavior are being investigated.

Introduction/ Literature Review

Gravity is a universal force on the Earth's surface and every known organism evolved and developed under the same gravitational force and the mechanical stress which it imposes. Because of this constant force, it is likely that many developmental processes rely upon gravity [10]. While all things develop under the influence of this force, it is difficult to determine which of these adaptions and mechanisms are gravity dependent. Because of this, research can manipulate and alter these forces in order to observe the impact on the development of physiological processes. There are two manners in which to manipulate these forces: by decreasing force, microgravity or hypogravity, or by increasing force, known as hypergravity. While the research in this thesis will center on hypergravity, the change of the force in either direction could potentially alter the development differently, so both have been previously studied.

The research I conducted used *Xenopus laevis,* commonly known as the African clawed frog, which is a mainly aquatic amphibian. Like all other amphibians, they have a three-chambered heart composed of two atria and one ventricle, distinct from the four-chambered of amniotes including humans. The muscle in the ventricle of the heart, the myocardium, pumps and circulates the blood and nutrients throughout the developing and adult body. Gravitational studies have been conducted on several model organisms including amphibians, birds, and mammals (i.e. frogs, chickens, and rats) [13, 14, 16]. While very different from each other, these organisms are comparable to a certain extent because they are all vertebrates and therefore have similar development.

While there are multiple options when choosing a suitable test subject, my research uses frogs for their convenience. Following mating, they release hundreds of eggs at a time, which supplies a large number of subjects at the same age with relatively similar genetics, removing variability. The embryos are also small so that an experiment with large numbers is possible. Furthermore, the time from gastrulation to feeding is a short period, allowing for more time studying embryos rather than waiting for development.

Along with convenience, there is also a large body of knowledge of the progression of embryonic, larval, and adult development. For my research, stages were determined by morphologic criteria for *Xenopus laevis* which has been assembled by Nieuwkoop and Faber [11]. The stages are not time-dependent, but rather based upon the changes in morphology. Each stage is give a chronological number from one to sixty-six, including development from the one-cell fertilized stage, to cleavage, gastrulation, neurulation and through all tadpole stages until adulthood [11].

Previous research has shown that early *Xenopus laevis* embryonic development is altered when subjected to a change in gravity. For example, when *Xenopus* eggs at gastrulation were subjected to microgravity via a simulator for 5 days, they showed a difference in morphology compared to the control group including malformations in head cartilages, structures derived from neural crest cells, which begin migration following gastrulation as well as many other abnormalities [12]. In an earlier study in which embryos were subject to microgravity shortly after fertilization (prior to gastrulation) for a short period, there were

many abnormalities as well, but morphology returned to normal following return to a 1G environment [3].

In studies involving hypergravity, such as those conducted by Remus and Wiens [13], embryos were subjected to forces of 7G or 10G for 5 days via centrifugation. Similar to those subjected to microgravity, these larvae showed asymmetries in the head cartilages. There was also evidence of a decrease in size. However, there did not appear to be any malformations more incapacitating in experimental embryos than in control embryos. They did observe that those subjected to hypergravity had a significantly shorter body length than control embryos. Remus and Wiens hypothesized that this may be the result of a reduced ability to deliver the nutrients in the yolk material because of insufficient cardiovascular circulation. They suggested that this weaker cardiovascular circulation was a result of the increased resistance, and imposed by hypergravity that it would lead to cardiac hypertrophy. It is this hypothesis that led me to the start of my research and thesis.

Cardiac hypertrophy is the increase in size of the myocardium or cardiac muscle. It is relatively common and arises in response to many cardiac stresses such as cardiac arrhythmias, myocardial infarction, hypertension, endocrine disorders, and mechanical load. At first, this occurs to compensate for these stresses and to maintain a necessary cardiac output. However, sustained periods of cardiac hypertrophy can lead to heart fatigue and eventually overall weakness and a decreased stroke volume [6, 9]. The heart is an important organ in the developing vertebrate embryo because it is the first functional organ and is a part of the

circulatory system, the first functioning unit [7]. The heart, as a part of the circulatory system, is the mechanism through which nutrients are distributed throughout the body.

Cardiac hypertrophy results in the increase of various molecules and the rate in which they are expressed by means of gene regulation. An increase in the occurrence of these factors can be an indication of cardiac hypertrophy. One of these markers is fibronectin, and its elevation results from the formation of scar tissue in the heart. Fibronectin is also responsible for influenicing collagen fibril orientation, thereby affecting cardiac compliance, something that hypertrophy decreases. Fibronectin can be detected and measured using antibodies as a means of determining presence and/or severity of cardiac hypertrophy. Collagen is another scar tissue elevated protein and can be similarly assessed. Yet another marker is ANF (atrial natriuretic factor) and is a volume-regulating hormone that is stored within the atria. It appears to be secreted by atrial myocytes when the cavity stretches, most often because of an increased blood volume, and it stimulates vasodilation [2]. Hypertension or an increased cardiovascular load can also result in an increase in the presence of ANF and can be an indication of cardiac hypertrophy [9].

Along with differences in cardiac development is a difference is swimming behavior and orientation. In vertebrates, it is the vestibular system, which controls the body, head, and eye posture, as well as swimming behavior. In a study using *Xenopus* embryos and hypergravity, the vestibular system was examined and it was noted that there were significant physiological and behavioral changes [1]. These effects lasted long after the termination of the exposure to experimental gravitational forces. One of these effects is the roll-induced vestibular reflex

(rVOR) and results in fictive swimming of the exposed tadpoles. This study will lead to research of orientation ability and swimming patterns because of an altered vestibular system.

Based upon the previous research of Remus and Wiens (2008) and Duchman and Wiens (2012), it has been shown that hypergravity will induce hypertrophy of the developing embryonic heart and will reduce overall body dimensions [4, 13]. Consequently, the purpose of this research and thesis is to study the effects of hypergravity on development of *Xenopus laevis* embryos and the developing heart, body, and behavior.

Materials and Methodology

Embryo Collection:

Embryos were produced by injecting an adult pair of *Xenopus laevis* frogs with human chorionic gonadotropin, which induced mating behavior and spawning, according to a previously established protocol [5, 15]. Fertilized embryos were sorted with aid of a binocular microscope based upon viability and arrival at mid-gastrulation (Nieuwkoop and Faber stage 8). Only viable, normal embryos of the appropriate stage were used for the experiments. Once sorted, the embryos were subjected to a 2% cysteine solution in 20% Steinberg's solution (SS), at pH 8.0, for 5 minutes to completely dejelly the embryos. Following the cysteine solution, the embryos were subjected to five washes on 10% SS for five minutes each. Following the final wash, embryos were sorted again, removing any that were deceased or damaged. Of the remaining viable embryos, a sample was randomly selected and placed into Costar 24-well trays, each containing 2 mL of freshly aerated 10% SS (pH 7.4) and a substratum pad of 300µL agarose gel (1% agarose in 10% SS). For each experiment there were two trays of control embryos and two trays of experimental embryos (48 of each group, 96 total per experiment).

Exposure to Hypergravity or Normal Gravity

This experiment was founded upon the basis of the mechanical stress that gravity imposes on the embryos and the effects of varying this stress by manipulating the level of gravity. To simulate hypergravity in the experimental groups, two trays of 24 embryos were placed in an Eppendorf 5810 R centrifuge (A-4-62 rotor) on the swinging trays of its rotor. The

trays were spun for 72 hours at 190 RPM, 220 RPM, 270 RPM, and 300 RPM to yield 7G, 10G, 15G, and 17G, respectively. The control group trays were taped to the top of the centrifuge to standardize vibration. The internal temperature of the centrifuge was also monitored and adjusted to provide consistency of control and experimental groups. Every 24 hours, the centrifuge was stopped for a minimal amount of time to replace SS with fresh, aerated SS to maintain water quality. The 72 hour stopping point was chosen because it is the approximate time required to reach Stage 45 (Nieuwkoop and Faber 9), the time for normal beginning of feeding. At this point, the neural crest cell derived head skeleton's mandibles and ceratohyals would begin to function and the yolk absorption would quickly diminish.

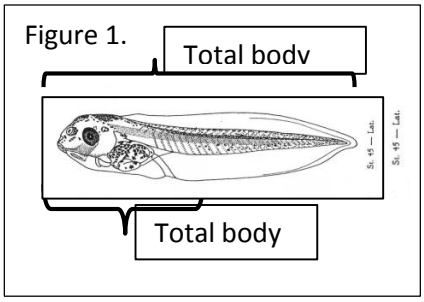
Post-Centrifugation

Following the completion of the 72-hour experiment, the centrifuge was stopped and all four trays (96 embryos) were removed from the centrifuge (either above or inside it). Any deceased embryos were discarded and mortality rates were recorded. General examination of all larvae in both groups was carried out to observe any differences between control and experimental embryos. A touch test to determine differences in responsiveness was performed by lightly prodding the tail, trunk, and head region of each embryo in sequence and recording the first touch to elicit a response. From each set of embryos, five were removed and flash frozen for future study of atrial natriuretic factor (ANF) and ten were fixed in 4% paraformaldehyde for various studies. Remaining embryos from each group were kept in separate 250mL dishes for future behavior studies and long-term hypergravity effect studies.

Behavior Studies

Remaining embryos were observed for 5 minutes daily to record abnormal and fictive swimming that may occur. During fictive swimming, embryos were upside down while swimming and unable to swim in a correct position. Embryos were also subjected to swimming orientation tests to observe differences in time. Each embryo was placed in a 50mL graduated cylinder, inverted twice, and then timed to determine ability and speed required to orient and swim to the bottom. Normally all embryos first seek the bottom following disorientation. The time required to reach the bottom was recorded. Following 2 months of behavior studies, all remaining embryos were fixed in 4% paraformaldehyde for embedment and sectioning to observe long-term effects of hypergravity at a later time. Each day of behavior studies, deceased embryos were discarded and were recorded to determine if there was a difference in mortality rates.

Figure 1.



Fixation and Embedment

All embryos fixed in paraformaldehyde were measured using a binocular dissecting microscope for snout-vent length as well total body length and recorded. Figure 1 demonstrates the span of these measurements. After fixation and measurements, embryos were dehydrated and embedded in 100% paraffin. This procedure is shown in Figure 2.

Figure 2.

Dehydration and Embedment Procedure

1. Deionized water: 5 minutes
2. 25% Ethanol: 5 minutes
3. 50% Ethanol: 5 minutes
4. 70% Ethanol: 5 minutes
5. 90% Ethanol: 5 minutes
6. 2x 100% Ethanol: 5 minutes
7. 1:1 Protocol: 100% Ethanol: 5 minutes
8. 100% Protocol for 15 minutes at 55-58°C
9. 1:1 Paraffin: Protocol overnight at 55-58°C
10. 2x 100% paraffin overnight at 55-58°C

Note: Protocol Safeclear II, Fisher Scientific

Sectioning and Immunostaining

Following embedment in paraffin, the embryos were individually mounted onto wooden blocks and sectioned at six µm and placed onto warm water on Tissue Section Adhesive (TSA) coated slides. Embryos were sectioned using a Reichert-Jung Histocut 820 microtome. The wax sections were placed upon watered TSA coated slides and the water were evaporated using a 42°C slide warmer to allow the sections to dry on the slides. Each slide was marked to note group (control or experimental), embryo number, and slide number to maintain organization. Once dried, the slides were deparaffinized and then passed through an immunostaining procedure as shown in Figure 3.

Figure 3.

Rehydration and Immunostaining Procedure

1. Xylene x2 for 2 minutes
2. 1:1 Xylene:EtOH for 2 minutes
3. 100% EtOH x2 for 2 minutes
4. 90% EtOH x1 for 2 minutes
5. 70% EtOH x1 for 2 minutes
6. 50% EtOH x1 for 2 minutes
7. Tap water x1 for 2 minutes
8. Hematoxylin x1 for 2 min
9. Running H_2O x1 in a beaker (sink) for 2 min
10. Acid Alcohol x1 for approx. 1.5 minutes
11. Standing H_2O x1 for 2 min
12. Ammonia solution x10 dips
13. Running H_2O for 5 min
14. 70% EtOH x1 for 2 min
15. Eosin for 15 seconds
16. 90% EtOH x1 for 2 min
17. 100% EtOH x2 for 2 min
18. Xylene x2 for 2 min
19. Add 3 drops GVA mount and coverslip

Dimensional Analysis of Sections

After sections were stained and the GVA-mounting medium (Zymed, Inc., San Francisco, CA) dried, the sections were viewed with a Leica DMIRE 2 microscope using brightfield and differential interference contract optics. Using image analysis software, (Image J, National Institutes of Health) the sectioned

embryos were analyzed and the transverse cross sectional area of the ventricle in profile was measured. Papillary muscle thickness and area was also measured and recorded. Multiple samples from each embryo were collected to find the average and to attempt to minimize error. The image software was recalibrated before each use to insure best measurements possible.

Statistical Analysis of Data

Once all measurements for the embryos were made, SAS (Statistical Analysis System) Software were used to conduct Shapiro-Wilk normality tests on all data sets. It was used to perform two-sample t-test on the corresponding control and experimental data sets. From each experiment, measurements were collected from hypergravity and control groups and recorded. Following data collection, a statistical standardization was completed in order to normalize differences between different experimental groups. This was achieved by first comparing and analyzing all control groups and finding an averaged measurement. Next, the average difference in size of a control group was calculated and recorded as a percentage. Finally, the difference in average size was applied to the corresponding experimental group for a normalized set of data across all experimental groups. Note that outliers remained in collected data but were not be used in calculations. Significance probabilities of all measurements were completed and recorded. In addition, the standard deviation was calculated for all groups and displayed in the appropriate figure.

Results

There were no significant differences in mortality rates between experimental and control groups of 7G, 10G, or 15G though there was a significant difference in experimental

group 17G. Groups 10G and 15G had very slight differences in mortality, while group 17G had an astounding result. Mortality was 4% among the control embryos but 100% among those as 17G.See Table 1 for mortality rates amongst experimental groups.

Table 1: Survival Rates of Control and Experimental Groups

	Control	7G	10G	15G	17G
Alive	230	92	45	42	0
Dead	10	4	3	6	48
Percent Survival	**95.8%**	**95.8%**	**93.8%**	**87.5%**	**0%**

Table 1 depicts the number of living and deceased individuals from control and experimental groups throughout research. Percent survival rate of populations is in the bottom row.

Embryo Touch Tests Responsiveness

There were statistically significant differences between control and each experimental group within the touch tests. For control groups, there was an average 90% response rate for the minimal tail touch and only 1% of individuals that did not respond to any physical stimuli. Experimental embryos showed 75%, 63%, and 43% response rates to the minimal tail touch test and 4%, 7% and 10% that did not respond to any stimuli for 7G, 10G and 15G, respectively.

Table 2: Embryo Touch Test Responsiveness

	Control	7G	10G	15G

Tail touch	91.4%	75.0%	63.6%	42.9%
Trunk	5.7%	16.7%	22.7%	35.7%
Head	1.9%	4.2%	6.8%	9.5%
No Response	1.0%	4.1%	6.9%	11.9%
Total:	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Table 2 depicts the responsiveness of embryos immediately following the termination of centrifugation. Each embryo was lightly touched in the order tail, trunk, and head. If the embryo responded (noted by a change in swimming direction or fictive swimming), the result was recorded and the embryo was not poked in any other location. Each embryo was touched until a response was recorded or there was 'no response' recorded.

Embryo Total Body Measurements

Statistically significant differences in overall body length were found between all experimental groups compared to the control group. A significant difference was also found between all experimental groups in total body length except for of those from the 10G to the 15G group as compared to each other. Embryo length was found, on average, to be longer in control embryos than those within all experimental group's averages.

Figure 4: Total Body Length

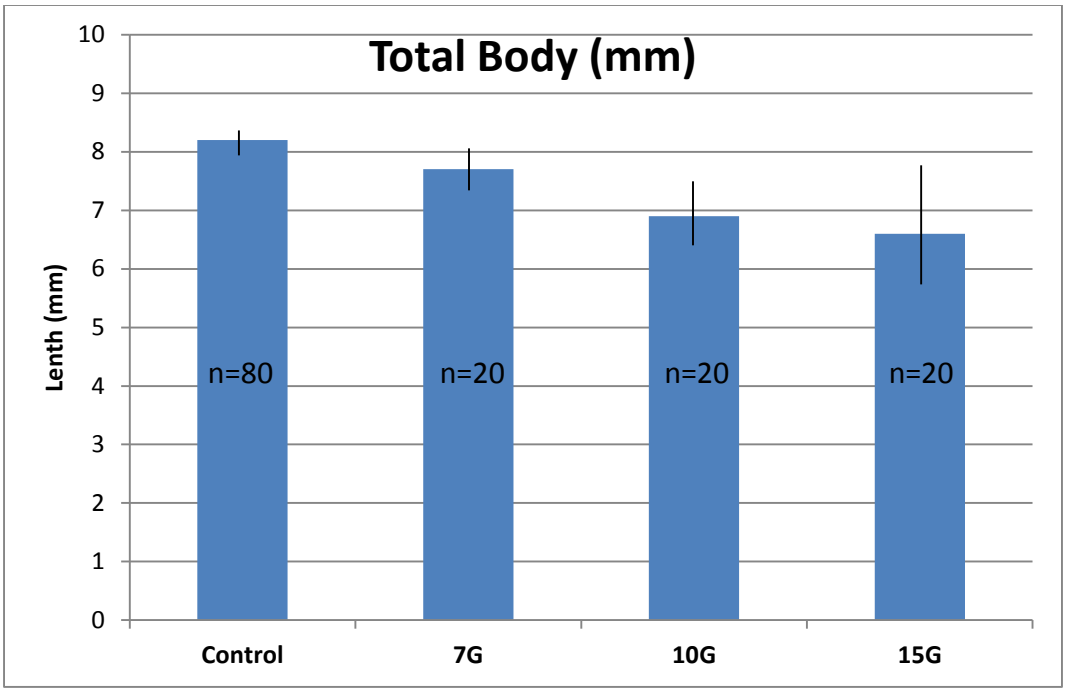


Figure 4. The total body length of embryos was reduced within all populations of experimental embryos as compared to the control groups.

Embryo Snout-to-Vent Body Measurements

Statistically significant differences in snout-to-vent length among experimental versus control groups was not established, except in the 15G versus control group. Within this group, the snout-to-vent length was larger in the experimental groups than in the control groups.

Figure 5: Snout-to-Vent Length

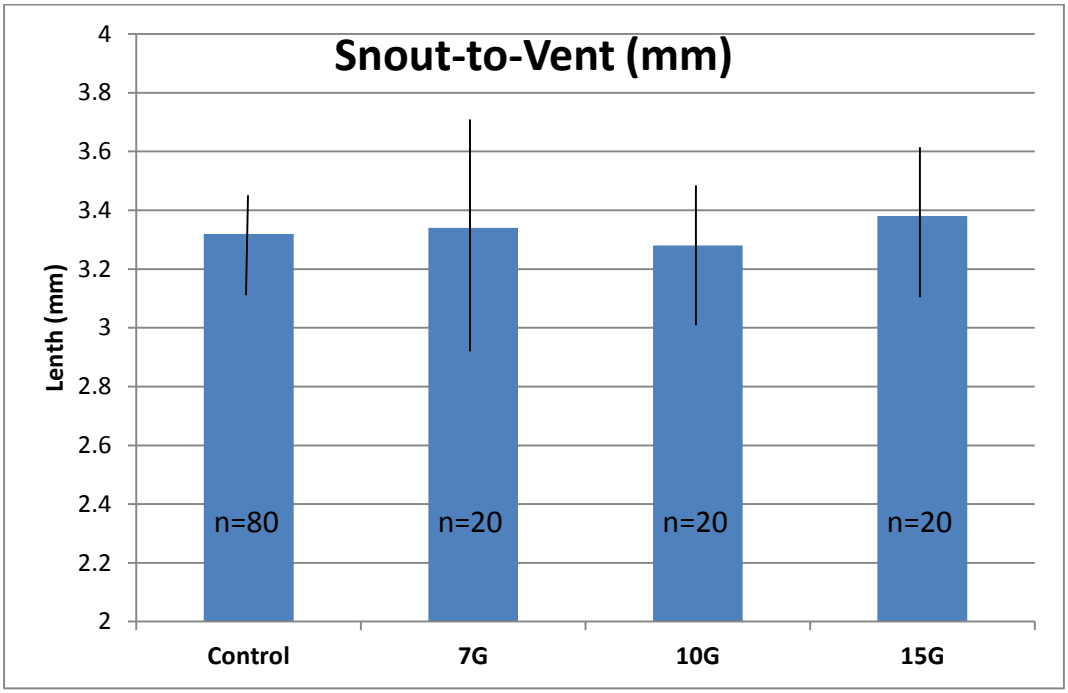


Figure 5. The snout-to-vent length was measured among all populations of control and experimental organisms. The results shown in graph 2 above are represented in millimeters.

Embryo Snout-Vent Measurement Comparison

The ratio of the above measurements was made by dividing the total body length by the snout-to-vent length to determine the percentage of the body that was made up by the anterior body region as compared to the whole. The results of these were all determined to be

statistically significant among all experimental groups as compared to the control group and demonstrated a trend line that increased in conjunction with the increased level of hypergravity. As the force of gravity increased, the overall body length decreased while the snout-to-vent length either stayed the same or increased resulting in an increase in the percentage of body composed of vital organs and a smaller percentage composed of tail region.

Figure 6: Snout-Vent Ratio

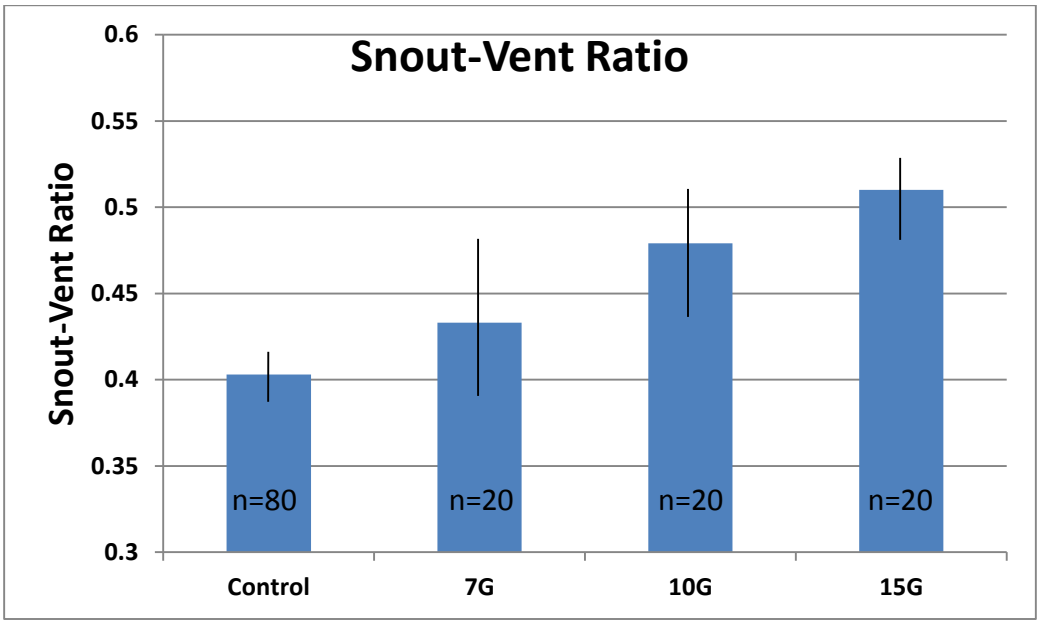


Figure 6. The overall percentage of the body composed of the head and trunk of the embryo increased in parallel with an increase in gravitational force during development. The above graph demonstrates this trend as a number out of 1. By multiplying by 100, the percentage of the body composed of the head and trunk region can be calculated.

Cross Sectional Width of Ventricle Wall

Embryos from each group were cross sectioned and stained. Statistically significant differences in ventricle wall thickness were not observed within the 7G versus control group.

n=80 n=20 n=20 n=20

Significant differences from control were found at 10G and 15G The ventricle wall thickness was larger in the experimental groups than in the control groups.

Figure 7: Ventricle Wall Thickness

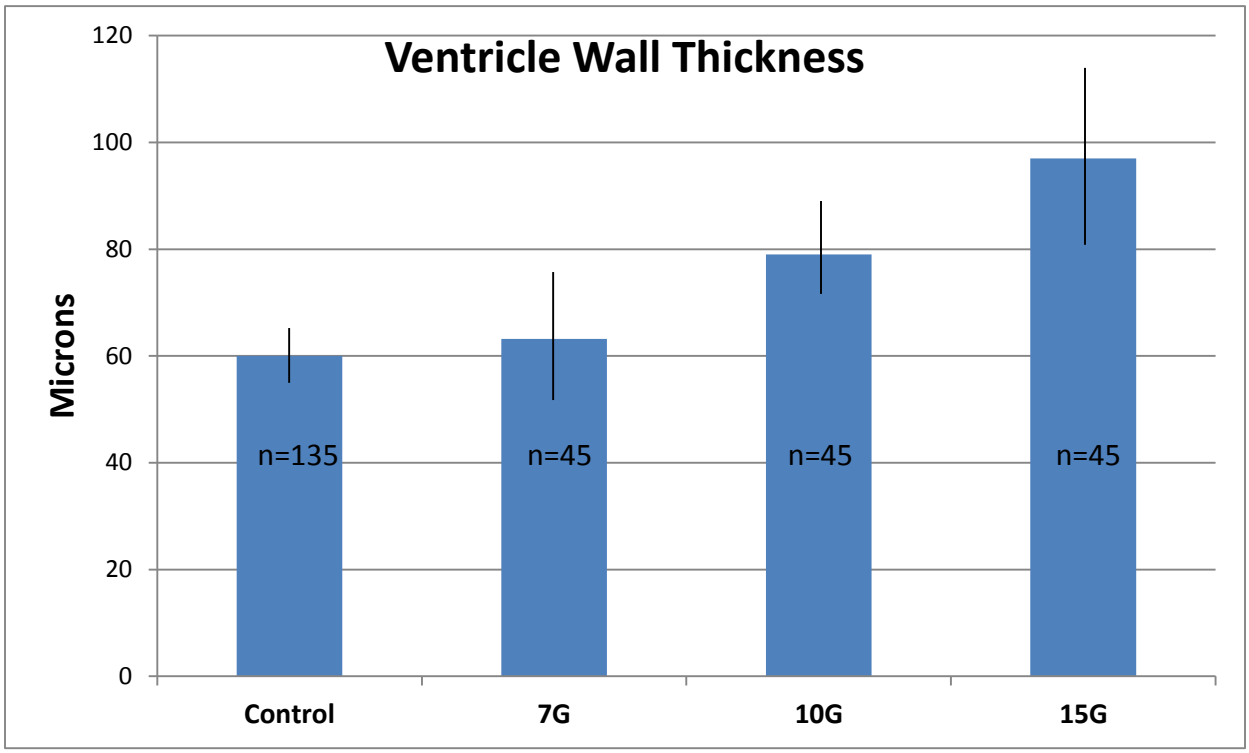


Figure 7. The ventricle wall thickness was measured among all populations of control and experimental organisms. The results shown in graph 4 above are represented in microns.

Cross Sectional Length of Cardiac Papillary Muscle

Embryos from each group were cross sectioned and stained. Statistically significant differences in papillary muscle length were established within the control and all experimental

groups. Within this group, the papillary muscle length was larger in the experimental groups than in the control groups.

Figure 8: Papillary Muscle Length

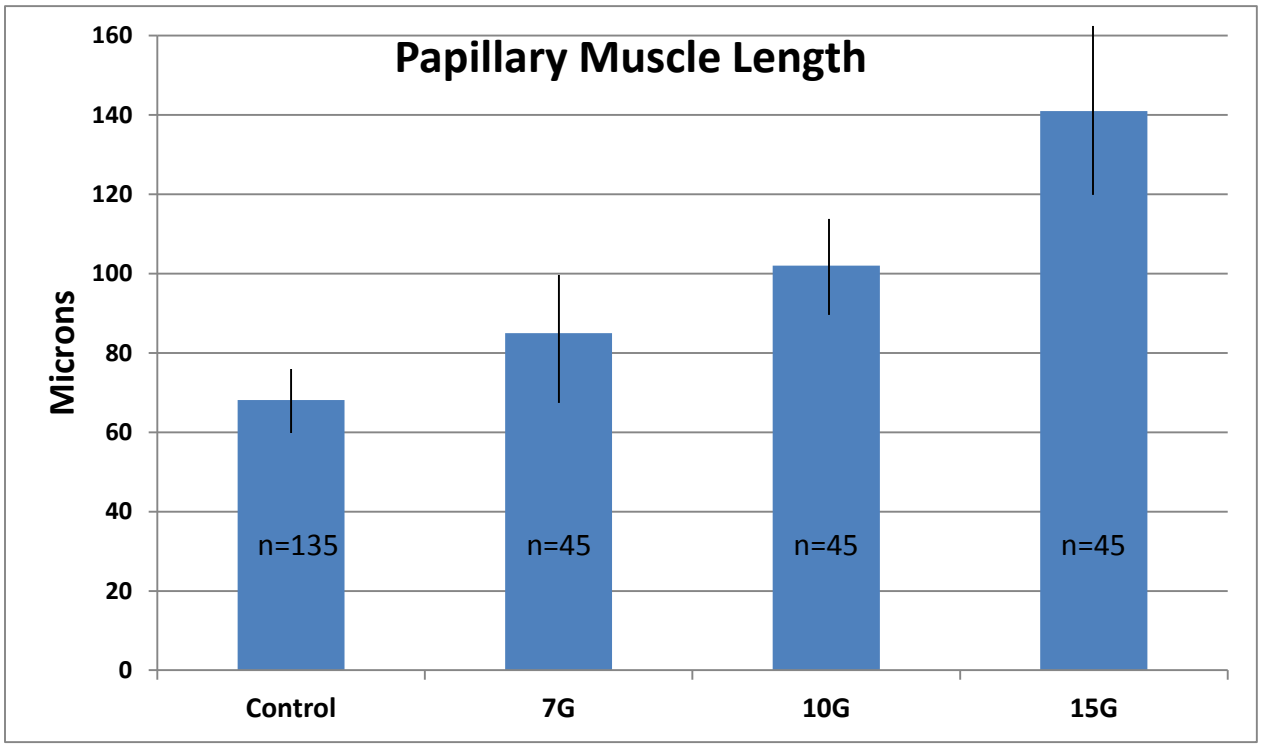


Figure 8. The papillary muscle length was measured among all populations of control and experimental organisms. The results shown in graph 5 above are represented in microns.

Cross Sectional Area of Cardiac Papillary Muscle

Embryos from each group were cross sectioned and stained. Statistically significant differences in papillary area were established within the control group versus all experimental

groups. Within this group, the papillary muscle area was larger in the experimental groups than in the control groups.

Figure 9: Papillary Muscle Area

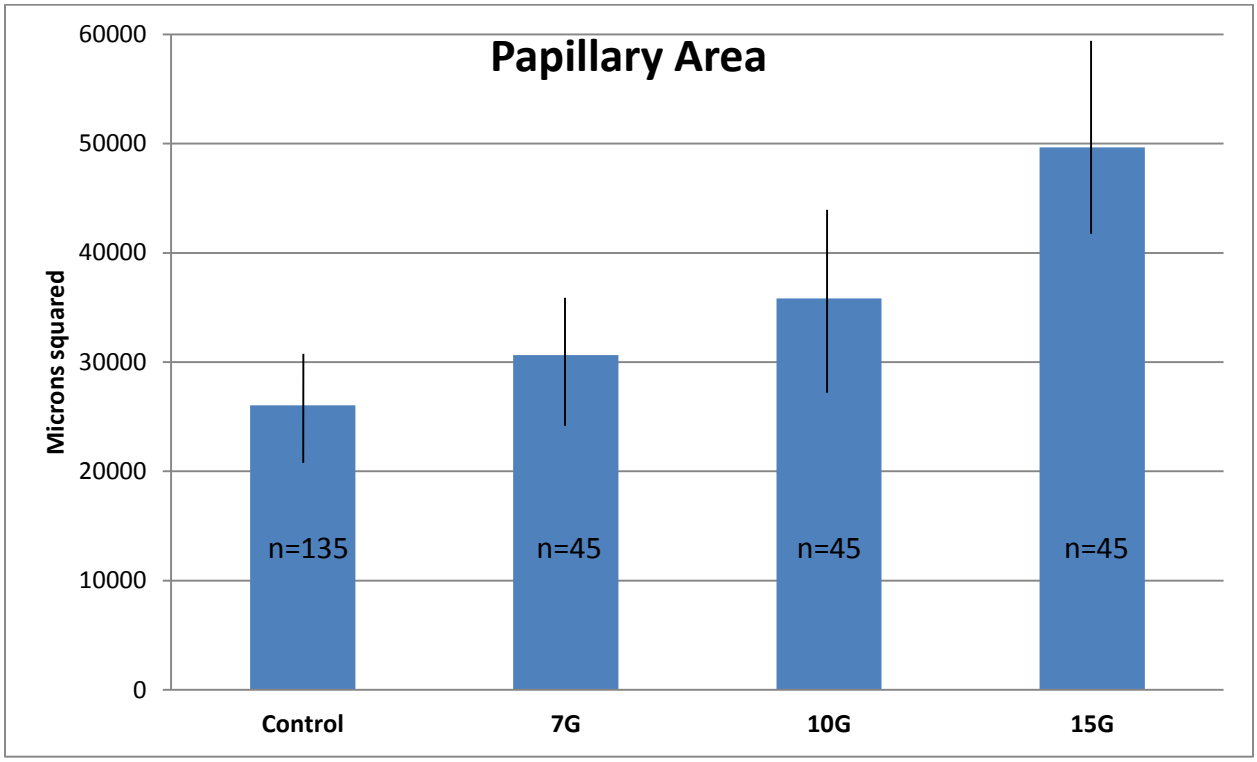


Figure 9. The papillary area was measured among all populations of control and experimental organisms. The results shown in graph 6 above are represented in microns squared.

Fictive Swimming

Embryos from control and 7G groups were observed for 5 minutes/day for 2 months. Those that exhibited the behavior were recorded and removed from the well to prevent double

counting. Statistically significant differences in number of fictive swimmers were established within the 7G versus control group. Within this group, the fictive swimming had a higher rate of occurrence in the experimental groups than in the control groups.

Figure 10: Fictive Swimming

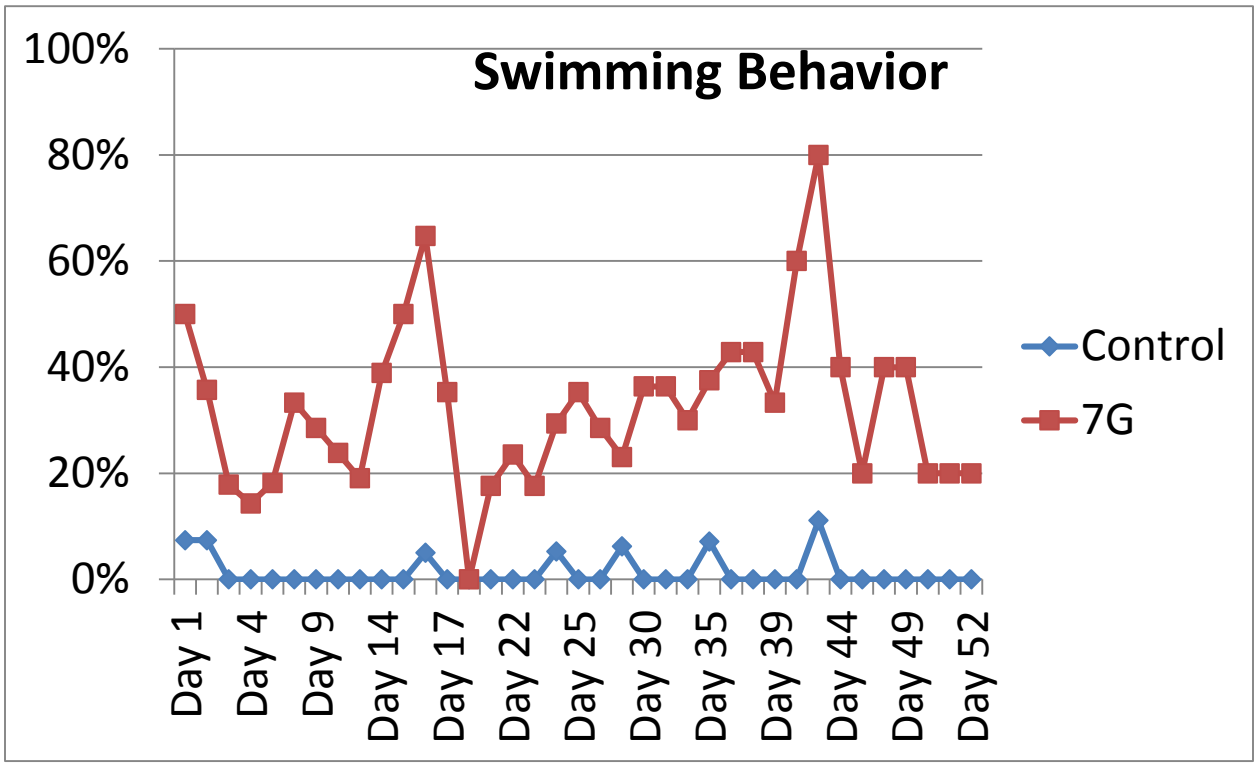


Figure 10. The above graph demonstrates the occurrence of fictive swimming among all populations of control and experimental organisms. The results shown in graph 7 above are represented as the percentage of total individuals within group.

Orientation Swimming Test

Embryos from each group were placed individually into a 50mL test tube and inverted twice. They were then observed and the time required to reach the bottom was recorded. Statistically significant differences in time were established within the 7G versus control group. Within this group, the time required to reach the bottom was longer in the experimental groups than in the control groups.

Figure 11: Orientation Swimming Times

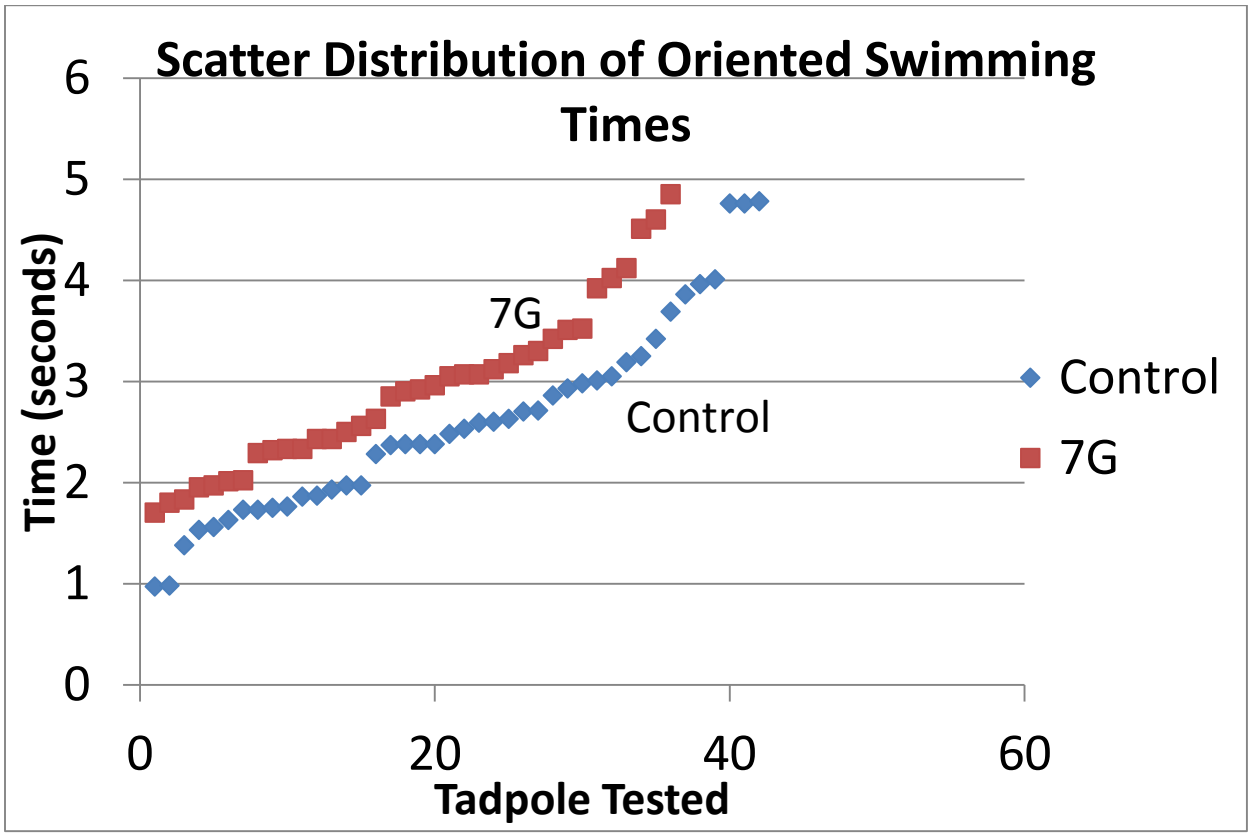


Figure 11. The snout-to-vent length was measured among all populations of control and experimental organisms. The results shown in graph 8 above are represented in seconds.

Discussion

Although there have been numerous studies that observe the effects of simulated hypergravity upon the developing amphibian heart, there have been very few studies that have focused specifically upon the difference of partial and total embryo heart size as well as the ratio of these components. The initiation of the beating heart is the vital component in the circulation of blood. Furthermore, the heart is responsible for the dispersion of nutrients throughout the body from the yolk mass via the developing circulatory system. The increase of gravitational force could add a resistance component to the dispersion of these nutrients and therefore place an increased load upon the heart. This increased workload and cardiac stress could therefore result in cardiac hypertrophy, a cardiac enlargement condition, in an attempt to maintain necessary cardiac output to supply nutrients to the developing body. This would result in an increase in size of the heart, particularly within the ventricle, as a response to this gravitational stress. The hindered ability of the heart to pump nutrients throughout the rest of the body may have an effect on the total body length of the developing organism in a negative manner as well as an enlargement effect upon the snout-to-vent length, the area in which the heart resides.

In this study, *Xenopus laevis* embryos were brought to gastrulation in standard (1G) gravity. The embryos were sorted for viability and exposed to simulated hypergravity via centrifugation. The initiation of gastrulation was necessary for this study in order to omit the effects hypergravity may have on earlier development, i.e. the development of signaling centers, blastomeres, and germ layers. There were a total of five studies performed at the following gravitational levels: two studies at 7G, one study at 10G, one study at 15G, and one

study at 17G for approximately 3 days until stage 45 when feeding begins. This experiment encompasses the neurulation and organogenesis stages through feeding.

The average total body length decline commensurately with increasing gravitational force. The snout-to-vent length measurements were not found to be statistically significant on their own since nearly all embryos had the same snout-to-vent length throughout all gravitational levels. However, the percentage anterior body length proved to be statistically significant throughout all experimental levels. This percentage of body that was composed of head and trunk grew larger as the level of gravity increased. This implies a decreased amount of body length composed of the tail indicating an inability of the hypertrophied heart to distribute nutrients throughout the entirety of the body, particularly within higher gravitational forces. These results matched the findings of Remus and Wiens [13].

There was a very high survival rate as compared to the previous research of Duchman and Wiens. The survival rate was greater than 85% within control and all experimental groups, indicating that the centrifugation did not cause death among embryos during development. However, the centrifugation at 17G resulted in complete mortality. This indicates the maximum stress load that the embryos can withstand between a successful 15G experiment and a certain death among 17G centrifugation.

There were many differences within embryo's hearts, as shown in the results. The ventricle wall thickness, papillary muscle length and area all increased linearly with gravitational force. This, taken together with body size and proportion data can be taken as convincing support of cardiac hypertrophy and insufficient circulatory support for growth.

The data collected throughout this study has provided new information and insight into the effect of hypergravity upon the developing heart and embryo as a whole. However, as time was limited, I was unable to take as many measurements of heart cross-sectional area as I would have liked to take. In future studies, the development of cardiac hypertrophy under the influence of hypergravity could be analyzed using the marker peptides atrial natriuretic factor (ANF) and fibronectin (FN). ANF is believed to be a cardiac hypertrophy modulator and therefore is theorized to be in occurrence at a higher level within individuals of a hypertrophied heart. FN has been found to participate in modulating the orientation of collagen and therefore myocardial compliance. Myocardial compliance is an important feature that is usually decreased in the presence of cardiac hypertrophy, and therefore would result in a differing level of FN. Furthermore, FN levels may also be higher in areas of cardiac damage, a symptom of cardiac hypertrophy, as it accumulates in these areas.

Throughout this study, I have concluded that *Xenopus laevis* suffers a change in heart and overall body formation in the presence of hypergravity. These results were consistent with my hypothesis that hypergravity increases the stress load upon the cardiac system and that the cardiac output is significantly altered in the presence of hypergravity due to the mechanical stress that it imposes. Therefore, the purpose of my research was fulfilled by this study.

Acknowledgements

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Appendix A

Embryo Body Length Measurements in micrometers

	Snout-Tail	Snout-Vent		Snout-Tail	Snout-Vent
Control			**7G**		
Embryo 1	8590	3330	Embryo 1	7750	3570
Embryo 2	8800	3180	Embryo 2	7790	3450
Embryo 3	8390	3310	Embryo 3	7970	3470
Embryo 4	8410	3160	Embryo 4	8310	3520
Embryo 5	8650	3260	Embryo 5	8210	3430
Average:	**8568.000**	**3248.000**		**8006.000**	**3488.000**
S-V/S-T	0.379085			0.435673245	
Std Dev:	**171.523**	**75.961**		**248.757**	**56.745**

	Snout-Tail	Snout-Vent		Snout-Tail	Snout-Vent
Control			**7G**		
Embryo 1	9563	3799	Embryo 1	9722	3847
Embryo 2	9422	3619	Embryo 2	7320	2729
Embryo 3	9659	3775	Embryo 3	7988	3581
Embryo 4	9745	3692	Embryo 4	9714	3880
Embryo 5	9734	4045	Embryo 5	9494	3834
Embryo 6	9049	3580	Embryo 6	8161	3914
Embryo 7	9740	3809	Embryo 7	9353	3647
Embryo 8	9849	3813	Embryo 8	9442	3829
Embryo 9	9714	3766	Embryo 9	9714	4068
Embryo 10	9867	3702	Embryo 10	8296	3212
Embryo 11	9950	3925	Embryo 11	9705	3906
Embryo 12	9722	3845	Embryo 12	9104	3904
Average	9667.8333	3780.83333	**Average**	9001.066667	3695.965833
S-V/S-T	0.3910735		**S-V/S-T**	0.410614205	
StDev	238.77903	127.110427	**StDev**	834.4086957	375.5071981

	Snout-Tail	Snout-Vent		Snout-Tail	Snout-Vent
Control			**10G**		
Embryo 1	6702	2911	Embryo 1	6562	2956
Embryo 2	7513	3136	Embryo 2	5903	3094
Embryo 3	7073	3021	Embryo 3	4842	2820
Embryo 4	7047	3128	Embryo 4	5837	2919
Embryo 5	6508	2872	Embryo 5	6493	2975
Embryo 6	7233	3135	Embryo 6	5779	2330
Embryo 7	7435	3012	Embryo 7	5938	2999
Embryo 8	6636	2836	Embryo 8	5700	3019
Embryo 9	7289	3079	Embryo 9	6627	3080
Embryo 10	6450	2881	Embryo 10	5580	3294
Embryo 11	7289	3291	Embryo 11	6298	3048
Embryo 12	7703	3049	Embryo 12	5647	3088
Average	7073.1667	3029.25	**Average**	5933.833333	2968.5
S-V/S-T	0.4282735		**S-V/S-T**	0.500266831	
StDev	413.09207	135.5488	**StDev**	505.328486	231.7124001

	Snout-Tail		Snout-Vent			Snout-Tail		Snout-Vent
Control					**15G**			
Embryo 1	7056		2731		Embryo 1	5470		3418
Embryo 2	6364		2658		Embryo 2	5560		3088
Embryo 3	7519		2818		Embryo 3	5485		2832
Embryo 4	6303		2704		Embryo 4	5522		2789
Embryo 5	7291		2992		Embryo 5	5776		2990
Embryo 6	7296		2508		Embryo 6	4520		2537
Embryo 7	6217		2790		Embryo 7	5608		2928
Embryo 8	7401		2837		Embryo 8	5427		3066
Embryo 9	7144		2820		Embryo 9	5433		2712
Embryo 10	7343		2598		Embryo 10	6292		2781
Embryo 11	7399		3009		Embryo 11	7490		3332
Embryo 12	7024		2990		Embryo 12	7667		3326
Embryo 13	7767		3077		Embryo 13	6677		2690
Embryo 14	6966		3143		Embryo 14	4328		3157
Embryo 15	7540		3364		Embryo 15	5915		3025
Embryo 16	7662		3266		Embryo 16	6245		2908
Embryo 17	6761		2903		Embryo 17	4400		3313
Embryo 18	7468		2309		Embryo 18	6457		2791
Embryo 19	6633		2788		Embryo 19	7755		3482
Embryo 20	7849		3192		Embryo 20	6888		3307
Average	7150.6		2875.3		Average:	5946.3		3024.0
S-V/S-T	0.4021079				**S-V/S-T**	0.508561059		
StDev	481.6		261.8		StDev	999.9		272.7

Appendix B

Embryo Heart Cross Sectional Area 10G

10G	Ventricle Length	Papillary Length	Papillary Area
1	200.9	457.3	38355.8
2	145.4	313.7	24818.5
3	182.4	366.5	50389.0
4	132.2	275.8	39860.0
5	111.9	305.7	44372.4
6	142.7	230.0	27074.7
7	151.5	283.7	28578.8
8	151.5	419.4	39107.9
9	149.8	303.1	37603.7
10	129.5	415.0	29330.9
11	130.4	257.3	28578.8
12	160.4	351.5	21810.2
13	117.2	435.2	35347.5
14	136.6	296.0	38355.8
15	81.9	406.2	36851.7
16	132.2	240.5	33091.3
17	123.3	403.5	57157.7
18	136.6	266.1	54901.5
19	128.6	419.4	42868.3
20	188.5	472.2	30835.1
21	160.4	374.4	30835.1
22	111.0	398.2	54149.4
23	97.8	404.4	20306.0
24	116.3	409.7	47380.7
25	118.9	326.0	33091.3
26	99.6	365.6	36851.7
27	84.6	318.1	24066.4
28	74.0	247.6	32339.2
29	90.7	205.3	24818.5
30	91.6	244.1	39860.0
31	107.5	277.5	27074.7
32	127.8	279.3	37603.7
33	127.8	240.5	48884.9
34	117.2	295.2	46628.6
35	90.7	315.4	33091.3
36	119.8	299.6	18049.8
37	75.8	291.6	33091.3

10G	Ventricle Length	Papillary Length	Papillary Area
39	137.4	262.6	20306.0
40	169.2	228.2	37603.7
41	145.4	241.4	41364.1
42	102.2	293.4	24818.5
43	106.6	215.9	33843.4
44	94.3	229.1	32339.2
45	93.4	198.2	39860.0
46	111.0	273.1	63174.3
47	117.2	267.0	27074.7
48	139.2	307.5	27074.7
49	84.6	273.1	26322.6
50	131.3	342.7	45124.5
51	140.1	277.5	36851.7
52	151.5	279.3	33091.3
53	130.4	340.1	44372.4
54	131.3	296.0	36099.6
55	145.4	333.9	38355.8
56	81.9	221.1	33843.4
57	118.9	267.0	46628.6
58	117.2	308.4	29330.9
59	118.9	370.0	26322.6
60	129.5	309.3	23314.3
61	111.9	393.0	35347.5
62	125.1	216.7	46628.6
63	104.0	209.7	49636.9
64	84.6	260.8	26322.6
65	74.0	272.2	
66	129.5	307.5	
67	94.3	251.1	
68	91.6	349.8	
69	99.6	296.9	
70	90.7	339.2	
71	113.7		
72	90.7		
73	86.3		
74	86.3		

Embryo Heart Cross Sectional Area 15G

15G	Ventricle Length	Papillary Length	Papillary Area
1	167.4	490.7	43620.3
2	184.1	519.8	43620.3
3	151.5	459.0	45876.6
4	127.8	511.9	71447.1
5	225.6	329.5	88744.8
6	192.1	653.7	51141.1
7	123.3	549.8	77463.7
8	163.0	288.1	50389.0
9	140.1	341.9	54901.5
10	129.5	593.0	27826.8
11	123.3	361.2	36099.6
12	165.6	425.6	66182.6
13	125.1	374.4	51893.2
14	148.0	331.3	41364.1
15	110.1	393.0	34595.4
16	118.9	437.9	59413.9
17	160.4	519.8	66934.6
18	155.9	568.3	78215.8
19	166.5	526.0	72951.2
20	140.1	389.4	55653.5
21	127.8	292.5	38355.8
22	118.9	346.3	38355.8
23	135.7	351.5	45124.5
24	135.7	327.8	33091.3
25	116.3	317.2	31587.1
26	97.8	325.1	26322.6
27	143.6	306.6	39107.9
28	108.4	366.5	47380.7
29	98.7	341.0	44372.4
30	96.0	332.2	42116.2
31	81.1	350.7	59413.9
32	72.2	331.3	60918.0
33	74.9	236.1	74455.4
34	60.8	310.1	87992.7
35	98.7	410.6	51141.1
36	83.7	266.1	25570.5
37	181.5	326.9	24818.5
38	127.8	379.7	36099.6
39	165.6	234.4	30083.0

15G	Ventricle Length	Papillary Length	Papillary Area
41	117.2	289.9	49636.9
42	118.1	291.6	48884.9
43	117.2	221.1	
44	99.6	387.7	
45	137.4	390.3	
46	149.8	306.6	
47	139.2	239.6	
48	172.7	246.7	
49	133.0	322.5	
50	86.3	267.0	
51	126.0	328.6	
52	159.5	410.6	
53	149.8	330.4	
54	152.4	200.9	
55	119.8	211.5	
56	141.9	265.2	
57	118.9	196.5	
58	133.9	221.1	
59	122.5	438.8	
60	82.8		
61	139.2		
62	94.3		
63	111.0		
64	137.4		
65	85.5		
66	107.5		
67	139.2		
68	135.7		
69	128.6		
70	129.5		
71	159.5		
72	139.2		
73	155.9		
74	148.9		
75	174.4		
76	126.9		
77	122.5		
78	141.0		

Appendix C

Orientation Swimming Time

	Control				7G	
Trial #	1	2	3	1	2	3
1	3.19	2.38	2.53	4.85	2.33	3.3
2	2.7	3.25	2.86	3.05	2.92	1.95
3	3.01	4.76	2.38	3.26	4.02	2.02
4	3.96	1.53	2.59	Outlier	Outlier	Outlier
5	1.76	4.76	1.93	1.97	3.18	4.6
6	1.75	1.38	3.05	1.8	2.33	2.85
7	2.28	2.38	2.71	2.43	2.9	2.56
8	1.56	2.37	1.97	3.52	2.32	4.12
9	2.98	4.78	4.01	3.12	3.92	1.83
10	1.87	Outlier	Outlier	3.07	3.07	2.63
11	3.42	2.6	2.63	2.5	2.43	2.29
12	1.63	0.97	1.73	3.42	2.01	2.96
13	Outlier	2.93	3.86	1.7	3.51	4.51
14	2.48	0.98	3.69			
15	1.97	1.86	1.73			
average	2.509167	2.840833	2.6875	2.890833	2.911667	2.968333
std. dev	0.756915	1.336092	0.751649	0.883253	0.65866	0.975284
		AVERAGE	**2.679167**		**AVERAGE**	**2.923611**

This Study by: Stacey Howes

Entitled: The Effects of Hypergravity on Development of the Heart and Behavior of *Xenopus laevis*

Has been approved as meeting the thesis requirement for the Designation University Honors with Distinction.

5-6-14
Date

Dr. Darrell Wiens, Honors Thesis Advisor, Biology

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